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An Examination of Microbial Populations in Different Brands and Flavors of Ice Cream

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AN EXAMINATION OF MICROBIAL POPULATIONS IN DIFFERENT BRANDS AND FLAVORS OF ICE CREAM

By
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ABSTRACT

MADELINE ANNE CAMPBELL: An Examination of Microbial Populations in Different Brands and Flavors of Ice Cream
(Under the direction of Dr. Colin Jackson)

Outbreaks of foodborne illness are of concern to many Americans. While pasteurization of ingredients and freezing eliminates most microbiological hazards, ice cream can act as a vehicle for pathogen transmission and be a cause of foodborne disease. This study examined the bacterial content of seven nationally distributed brands of ice cream and one local farmer's market brand. The aims of the study were to (1) determine if there was a higher bacterial content in chocolate and strawberry ice cream compared to vanilla ice cream, potentially because of more ingredients and more opportunities for bacterial contamination in those flavors, and (2) determine if the local farmer's market ice cream contained a greater number of bacteria than the commercial brands, possibly because of reduced processing and less regulation. Ice cream samples were incubated on tryptic soy agar and milk agar plates, the number of colony forming units was determined, and representative cultures from each sample were identified using 16S rRNA gene sequencing. While more bacteria were found in chocolate ice cream than strawberry or vanilla, high variability in bacterial counts between brands meant that differences between flavors were not statistically significant ($p=0.15-0.20$). In terms of brand, the greatest number of bacteria were found in samples from the farmer's market and the bargain bulk brand, both of which gave counts of >30 million bacteria per pint container. The lowest bacterial counts were found in brands that had plastic seals around the rim of the container, suggesting that packaging style is an important factor. *Bacillus cereus,* a common cause of food poisoning, was identified in

all brands except for the farmer's market brand, while *Escherichia coli* was identified in three brands.

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INTRODUCTION

In recent years, food safety has become an increasing point of concern for many Americans. The Centers for Disease Control and Prevention estimates that one out of six Americans, or 48 million people, fell ill from a foodborne illness in 2011. Over 125,000 people required hospital care, and 3,000 cases were fatal (Centers for Disease Control and Prevention 2015). There are more than 200 known foodborne diseases, the most common of which are caused by just four types of bacteria: *Campylobacter, Salmonella, Shigella,* and *Escherichia coli* (Nestle 2003). The shift towards industrial food production has caused outbreaks of foodborne illnesses caused by these and other pathogens to increase. Our current system revolves around economic efficiency, and while this results in the large-scale production of food at a low cost, manufacturers are not always able to prevent the introduction of bacterial pathogens into their product.

Ice cream is one of many food products that can potentially act as a vehicle for pathogen transmission. The United States is the world's largest producer of ice cream, and ice cream is found in over 90% of US households. Nearly a tenth of the milk produced in the U.S. is used for the manufacture of ice cream (Clarke 2011). Pasteurization of ice cream or its ingredients likely eliminates most microbiological hazards, and freezing also controls microbial growth (Andreasen and Nielsen 1998). However, long storage times, a high nutrient content, and an almost neutral pH mean that ice cream remains susceptible to the introduction and subsequent growth of bacteria (Champagne et al. 1994; Tekins 2000).

There have been a number of recalls of ice cream products because of potential bacterial contamination. For example, in September 1994, a nationally distributed brand of ice cream (Schwan's) was recalled due to an increase in infections of *Salmonella enteritidis*, the most frequently reported type of *Salmonella* to cause disease in humans, associated with consumption of that product. At that time, this was the largest ever outbreak of *S. enteritidis* gastroenteritis in the United States, with an estimated 29,100 people falling ill in Minnesota alone, and 224,000 people becoming infected nationwide. The cause of contamination was traced to ice cream premix that had been contaminated during transportation in tanker trailers that had been used to carry non-pasteurized liquid eggs. Eggs and egg containing products are highly susceptible to contamination by bacteria, especially species of *Salmonella* (Hennessey 1996).

Milk is another ingredient of ice cream that is vulnerable to bacterial contamination (Marjan 2014), and flavored ice cream may also contain other ingredients (fruits, berries etc.) that could harbor pathogens. While there have been only a limited number of studies on the impact of flavor on the bacterial load in ice cream, a study conducted in the late 1940s compared bacterial counts in chocolate and vanilla ice cream from two companies in Indianapolis (Parker and Leander 1947). The scientists conducting the study believed that ice cream was a reservoir for undesirable milk and that chocolate flavored ice cream, because of its dark color and intense flavor, could cover up the presence of inferior ingredients more so than vanilla. Chocolate ice cream from one of the two companies tested contained three times as many culturable bacteria as vanilla, suggesting the use of ingredients with a higher bacterial content in the manufacture of chocolate ice cream. Comparing the bacterial content of vanilla ice cream to that of good market milk showed that ice cream was inferior in all points of comparison, including the number of staphylococci, which likely originated from unsterile processing equipment (Parker and Leander 1947). Microscopic examination showed that staphylococci, tetrads, and sarcinae were the most common bacterial cell types found in the ice cream samples and most likely entered the milk product from fat and casein residues found in crevices of utensils and machinery. Diplococci were also found, which the authors concluded as meaning the use of non-fresh milk during ice cream production (Parker and Leander 1947).

More recent studies have been conducted to determine the microbiological population of ice cream, although most have occurred overseas. A 2006 study was designed to determine the bacteriological quality and antibiotic susceptibility of 160 ice cream samples purchased in Tripoli, Libya. Many of the samples, particularly ones collected from the smaller manufacturers, contained bacteria that were resistant to one or more antibiotics. Resistance to a single antibiotic was found in bacteria obtained from over 80% of the samples tested and multiple-drug resistance was found in 25% (El-Sharef 2006). Another study conducted in 2006 in Rio de Janeiro, Brazil, examined the microbiological quality of 60 ice cream samples of various flavors from three commercial brands. The high level of bacterial contamination found was believed to reflect unhygienic conditions in the manufacturing and storage of this ice cream (De Farias 2006). More recently, a 2013 study, conducted in Samsun, Turkey, tested 100 samples of ice cream for the prevalence of enterotoxigenic and methicillin-resistant *Staphylococcus aureus* (Gucukoglu et al. 2013). This bacterium, which can cause nausea, vomiting, abdominal cramps, exhaustion, and even hospitalization, was found in 18 of the 56 (32%) fruit flavored ice cream samples tested, 4 of the 32 (12.5%) vanilla samples tested, and just 1 of the 12 (8%) chocolate samples tested (Gucukoglu et al. 2013), suggesting that flavor can have an important impact on the bacterial content of ice cream.

The aim of this study was to build upon this prior work and investigate the bacterial content of seven nationally distributed brands of ice cream in the United States, along with one local farmer's market brand from Clarksdale, Mississippi. My hypotheses were that (1) there would be higher bacterial counts in chocolate and strawberry ice cream compared to vanilla ice cream, because of increased numbers of ingredients (and therefore more likelihood of bacterial contamination) in those flavors, and (2) the farmer's market local ice cream would contain a greater number of bacteria than the commercial brands, because of reduced processing and less regulation.

METHODS

Sample Collection

Commercial ice cream was purchased from various stores in Oxford, MS, between August and November 2014. Three flavors (chocolate, strawberry, and vanilla), of each the following brands were obtained: Ben and Jerry's, Blue Bell, Blue Bunny, Bryers, Hagen Daaz, Kroger Party Pail, and Yarnell's. In addition, chocolate and vanilla flavors of Sweet Magnolia brand ice cream (a local brand from Clarksdale, MS) were also purchased. A summary of the ice cream samples is shown in Table 1. Immediately after purchasing, the ice cream was brought to the laboratory and stored unopened and sealed in the freezer (-20 °C) until experimentation.

Determination of Bacterial Counts within Ice Cream Samples

Bacterial counts in the ice cream samples were determined using Tryptic Soy Agar (TSA) and Milk Agar (MA) plates. TSA consisted of 28 g of TSA premix (consisting of 10.5 g pancreatic digest of casein, 3.5 g papaic digest of soybean, 3.5 g sodium chloride, and 10.5 g agar) and 700 mL H_2O; MA consisted of 3.5 g tryptone, 1.75 g yeast extract, 0.7 g dextrose, 0.7 g powdered skim milk, and 10.5 g agar combined with 700 mL H_2O. Bottles of agar were heated (microwaved) for 3-4 minutes, and then autoclaved (121 °C, 15 min) to sterilize. After agar had cooled to 53 °C, plates were poured following aseptic technique, allowed to cool, and refrigerated until use the following day.

Table 1: Brands and flavors of each ice cream sample examined for microbial populations as part of this study.

Brand	**Flavor**	**Store Acquired**	**Packaging**
Ben and Jerry's	Chocolate Therapy	Wal-Mart	473 mL
Ben and Jerry's	Strawberry Cheesecake	Wal-Mart	473 mL
Ben and Jerry's	Vanilla	Wal-Mart	473 mL
Blue Bell	Chocolate	Kroger	473 mL
Blue Bell	Strawberry	Kroger	1.89 L
Blue Bell	Vanilla	Kroger	473 mL
Blue Bunny	Chocolate	Kroger	1.65 L
Blue Bunny	Strawberry	Kroger	1.65 L
Blue Bunny	Vanilla	Kroger	1.65 L
Bryers	Chocolate	Wal-Mart	1.41 L
Bryers	Strawberry	Wal-Mart	1.41 L
Bryers	Vanilla	Wal-Mart	1.41 L
Hagen Daaz	Chocolate	Kroger	106 mL
Hagen Daaz	Strawberry	Kroger	414 mL
Hagen Daaz	Vanilla	Kroger	414 mL
Kroger Party Pail	Chocolate	Kroger	3.79 L
Kroger Party Pail	Strawberry	Kroger	3.79 L
Kroger Party Pail	Vanilla	Kroger	3.79 L
Sweet Magnolia	Chocolate	Farmer's Market	473 mL
Sweet Magnolia	French Vanilla	Farmer's Market	473 mL
Yarnell's	Chocolate	Wal-Mart	1.66 L
Yarnell's	Strawberry	Wal-Mart	1.66 L
Yarnell's	Vanilla	Wal-Mart	1.66 L

Approximately 1 g of ice cream (each brand, each flavor) was mixed with 9 mL sterile dilute yeast extract buffered solution and serially diluted ten-fold to yield dilutions of 10^{0} (the original solution), 10^{-1}, and 10^{-2}. 0.1 mL of each dilution was then pipetted onto each plate type (TSA and MA) spread evenly over the plate surface using an ethanol sterilized glass spreader. Three replicate plates per dilution per sample were made. Plates were labeled and incubated at 37 °C. The number of bacterial colonies on each plate was determined after 48 h and again after 7 d to allow for enumeration of slower growing bacteria.

Identification of Numerically Dominant Colonies

Samples of representative colonies from each sample were collected using sterile loops, mixed in sterile H_2O and centrifuged at 5,000 x g for 5 minutes. The supernatant was then removed and cell pellets frozen (-20 °C) for subsequent DNA extraction. At that time, the pellet of each sample was allowed to thaw to room temperature, and DNA extracted using a Mo Bio Ultraclean Microbial DNA Isolation Kit following the detailed protocol supplied by the manufacturer (Mo Bio Laboratories, Carlsbad, CA). The presence of DNA was verified by electrophoresis in agarose gels.

The V4 region of the bacterial 16S rRNA gene from the DNA from each colony was amplified and subject to paired end Illumina MiSeq next generation sequencing. Sequence data was processed and assembled into fasta files which were then compared to those in the GenBank database (BLAST searches in November 2015) to identify bacterial populations.

RESULTS

Bacterial Counts

After incubating the plates for 7 d, visible bacterial colonies on each agar plate were counted. Bacterial counts were then calculated in order to express the number of bacteria as the number of colony forming units (CFUs) per mL of ice cream and as the number of CFUs present in a typical pint (473 mL) of ice cream (Table 2). The greatest bacterial counts were seen in samples from the Kroger Party Pail and Sweet Magnolia brands, both of which gave samples with over 30 million CFUs per pint container (Fig. 1). In each case, both TSA and MA plates yielded high numbers of colonies for the chocolate flavor, which had 2-3 orders of magnitude more CFUs than strawberry or vanilla. The next highest bacterial counts were seen in samples of Blue Bell brand ice cream. The strawberry TSA and MA plates yielded the highest number of colonies–over 30,000 fold more than either the chocolate or vanilla flavors (Fig. 2). Ben and Jerry's vanilla MA plate yielded the most CFUs, but the TSA plates for the strawberry and vanilla flavors did not yield visible colonies. Yarnell's and Hagen Daaz had low and similar CFU counts across all flavors (Fig. 3). Blue Bunny brand had several plates that did not yield viable colonies and had low counts in the plates that yielded colonies (Fig. 4).

The lowest numbers of bacteria were found in Bryers brand ice cream with very low counts across all three flavors (Fig. 4). There was generally no consistent pattern in terms of one type of medium yielding more colonies than the other, as when all brands were compared, counts on MA and TSA were similar (Fig. 5). Averaging all brands, it appeared that the chocolate flavors yielded the most CFUs and the vanilla flavors

Table 2: Bacterial CFU counts in samples of ice cream as determined using tryptic soy agar (TSA) and milk agar (MA) plates. Counts are expressed per mL and per 473mL (1 pint), and are mean values from 3 replicate plates per sample/plate type.

Brand	**Flavor**	**Media**	**CFU/mL**	**CFU/pint**
Ben and Jerry's	Chocolate	MA	8.80×10^{1}	4.15×10^{4}
Ben and Jerry's	Chocolate	TSA	8.80×10^{1}	4.15×10^{4}
Ben and Jerry's	Strawberry	MA	5.50×10^{1}	2.60×10^{4}
Ben and Jerry's	Strawberry	TSA	0	0
Ben and Jerry's	Vanilla	MA	1.44×10^{2}	6.80×10^{4}
Ben and Jerry's	Vanilla	TSA	0	0
Blue Bell	Chocolate	MA	4.65×10^{2}	2.20×10^{5}
Blue Bell	Chocolate	TSA	1.37×10^{2}	6.46×10^{4}
Blue Bell	Strawberry	MA	6.64×10^{3}	3.14×10^{6}
Blue Bell	Strawberry	TSA	1.95×10^{3}	9.22×10^{5}
Blue Bell	Vanilla	MA	1.15×10^{2}	5.43×10^{4}
Blue Bell	Vanilla	TSA	1.44×10^{2}	6.80×10^{4}
Blue Bunny	Chocolate	MA	1.45×10^{2}	6.86×10^{4}
Blue Bunny	Chocolate	TSA	0	0
Blue Bunny	Strawberry	MA	0	0
Blue Bunny	Strawberry	TSA	1.17×10^{2}	5.55×10^{4}
Blue Bunny	Vanilla	MA	4.30×10^{1}	2.02×10^{4}
Blue Bunny	Vanilla	TSA	0	0

Bryers	Chocolate	MA	2.40×10^{1}	1.15×10^{4}
Bryers	Chocolate	TSA	9.70×10^{1}	4.60×10^{4}
Bryers	Strawberry	MA	0	0
Bryers	Strawberry	TSA	2.60×10^{1}	1.25×10^{4}
Bryers	Vanilla	MA	4.90×10^{1}	2.34×10^{4}
Bryers	Vanilla	TSA	2.50×10^{1}	1.17×10^{4}
Hagen Daaz	Chocolate	MA	8.30×10^{1}	3.94×10^{4}
Hagen Daaz	Chocolate	TSA	1.94×10^{2}	9.20×10^{4}
Hagen Daaz	Strawberry	MA	1.10×10^{2}	5.23×10^{4}
Hagen Daaz	Strawberry	TSA	7.40×10^{1}	3.48×10^{4}
Hagen Daaz	Vanilla	MA	1.21×10^{2}	5.73×10^{4}
Hagen Daaz	Vanilla	TSA	6.10×10^{1}	2.87×10^{4}
Kroger Party Pail	Chocolate	MA	8.81×10^{4}	4.17×10^{7}
Kroger Party Pail	Chocolate	TSA	1.10×10^{5}	5.20×10^{7}
Kroger Party Pail	Strawberry	MA	3.10×10^{1}	1.47×10^{4}
Kroger Party Pail	Strawberry	TSA	3.10×10^{1}	1.47×10^{4}
Kroger Party Pail	Vanilla	MA	5.50×10^{1}	2.58×10^{4}
Kroger Party Pail	Vanilla	TSA	2.70×10^{1}	1.29×10^{4}
Sweet Magnolia	Chocolate	MA	6.62×10^{4}	3.13×10^{7}
Sweet Magnolia	Chocolate	TSA	7.59×10^{4}	3.59×10^{7}
Sweet Magnolia	Vanilla	MA	5.51×10^{2}	2.60×10^{5}
Sweet Magnolia	Vanilla	TSA	5.80×10^{2}	2.74×10^{5}

Yarnell's	Chocolate	MA	3.53×10^2	1.67×10^5
Yarnell's	Chocolate	TSA	1.92×10^2	9.10×10^4
Yarnell's	Strawberry	MA	1.48×10^2	6.99×10^4
Yarnell's	Strawberry	TSA	3.38×10^2	1.60×10^5
Yarnell's	Vanilla	MA	1.36×10^2	6.41×10^4
Yarnell's	Vanilla	TSA	1.08×10^2	5.13×10^4

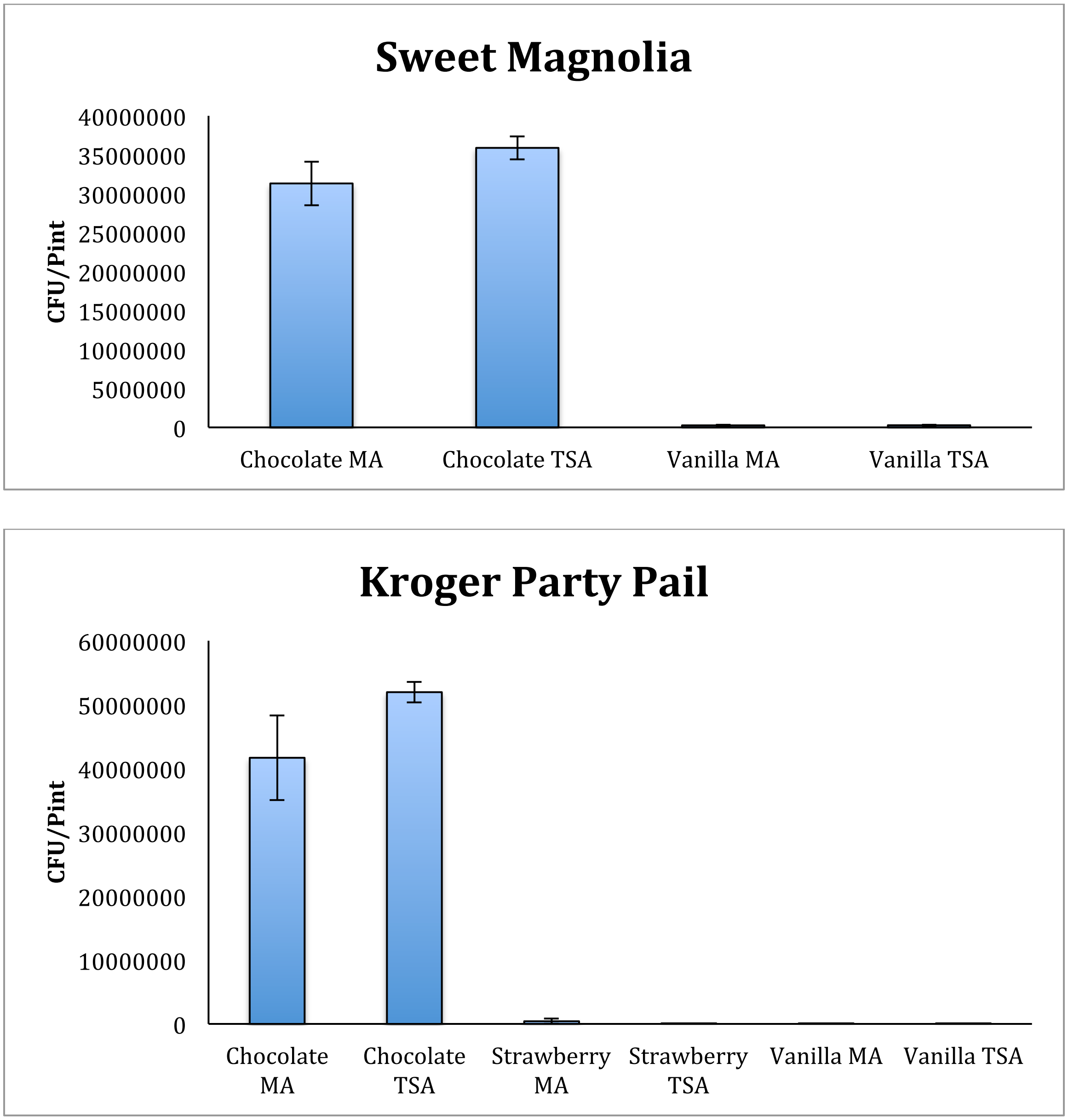
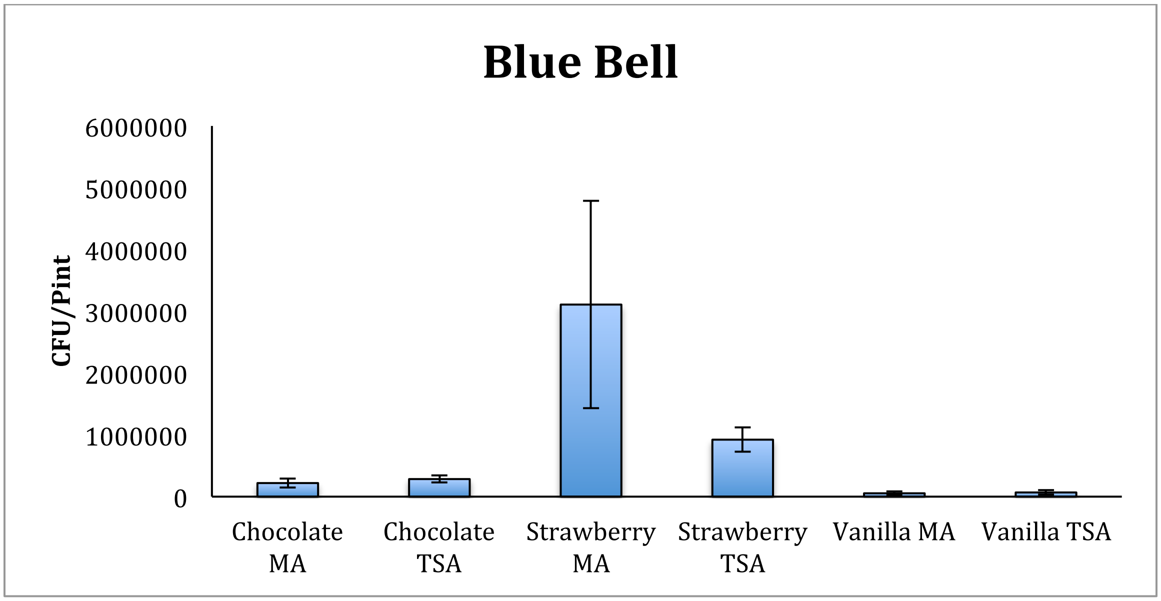


Figure 1: Bacterial counts in samples of two different flavors of Sweet Magnolia and three different flavors of Kroger Party Pail ice cream as expressed as CFU per pint carton. Counts were obtained using milk agar (MA) and tryptic soy agar (TSA) plates and are means (+/- SE) of three plates.



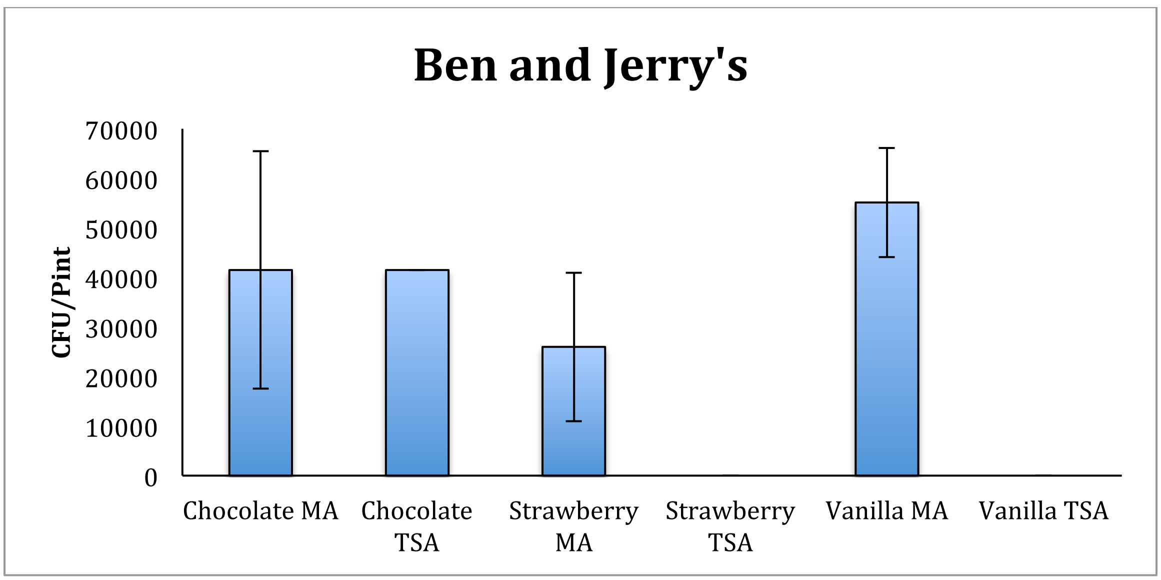


Figure 2: Bacterial counts in samples of three different flavors of Blue Bell and Ben and Jerry's ice cream as expressed as CFU per pint carton. Counts were obtained using milk agar (MA) and tryptic soy agar (TSA) plates and are means (+/- SE) of three plates.

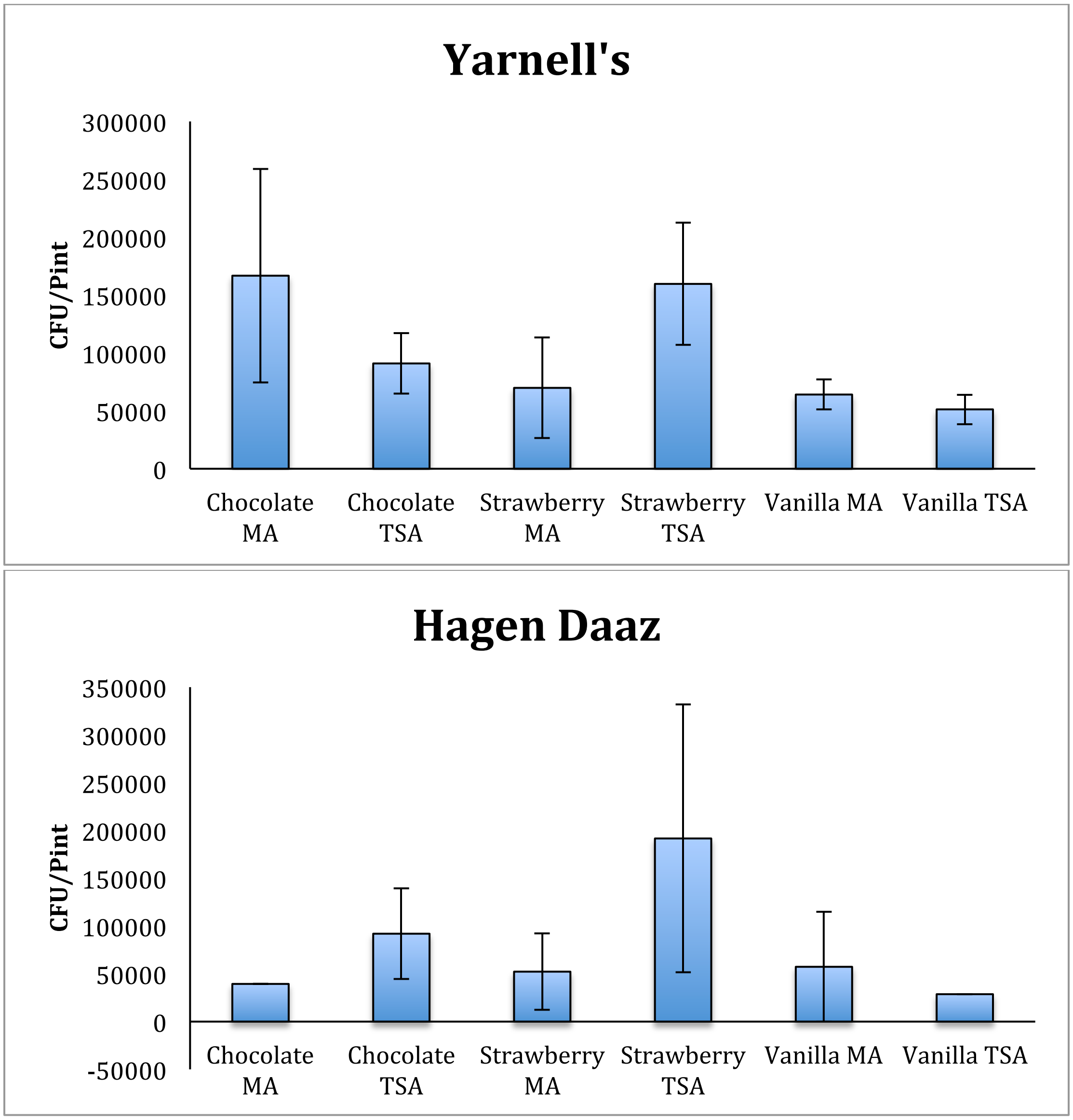


Figure 3: Bacterial counts in samples of three different flavors of Yarnell's and Hagen Daaz ice cream as expressed as CFU per pint carton. Counts were obtained using milk agar (MA) and tryptic soy agar (TSA) plates and are means (+/- SE) of three plates.

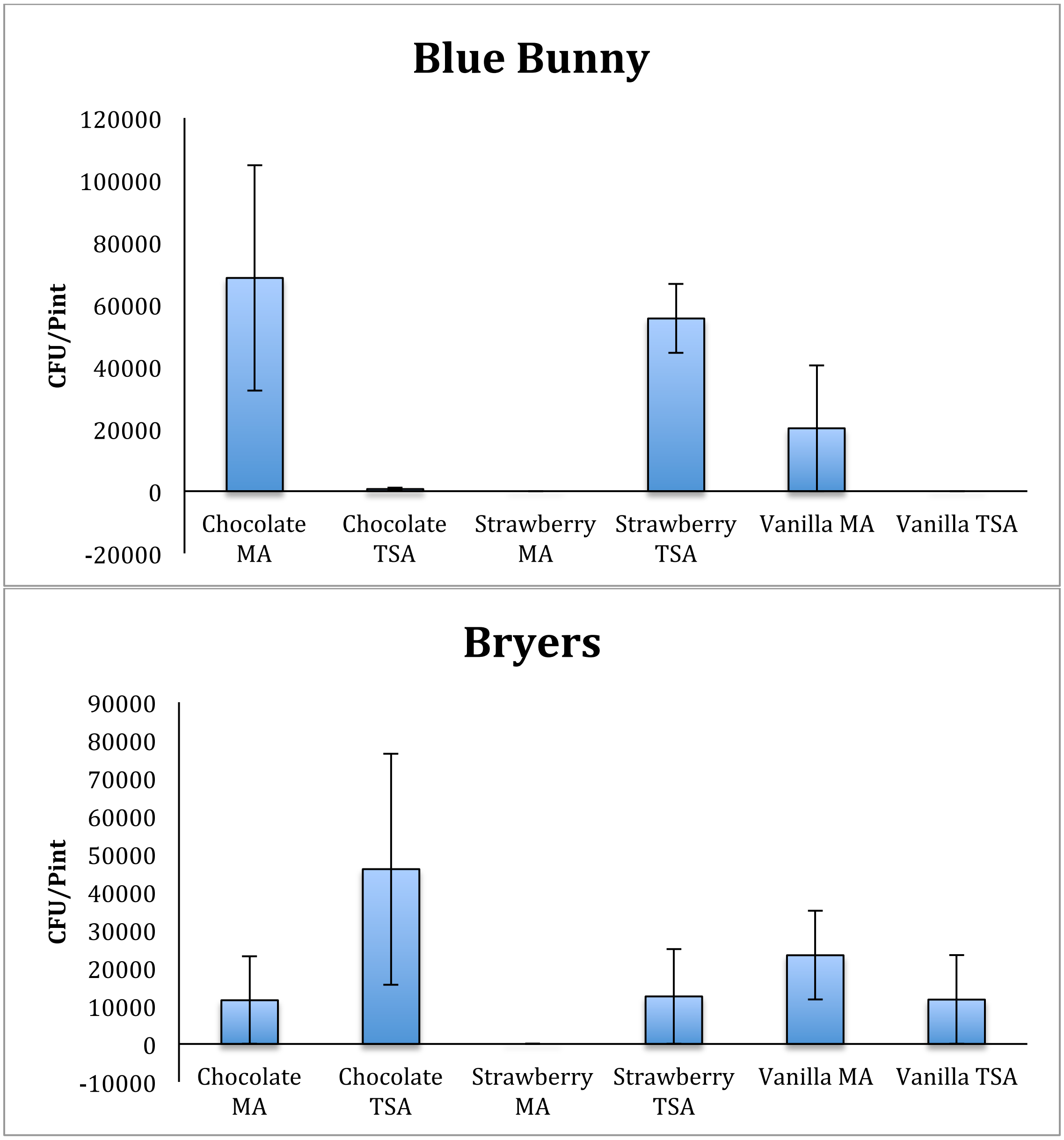


Figure 4: Bacterial counts in samples of three different flavors of Blue Bunny and Bryers ice cream as expressed as CFU per pint carton. Counts were obtained using milk agar (MA) and tryptic soy agar (TSA) plates and are means (+/- SE) of three plates.

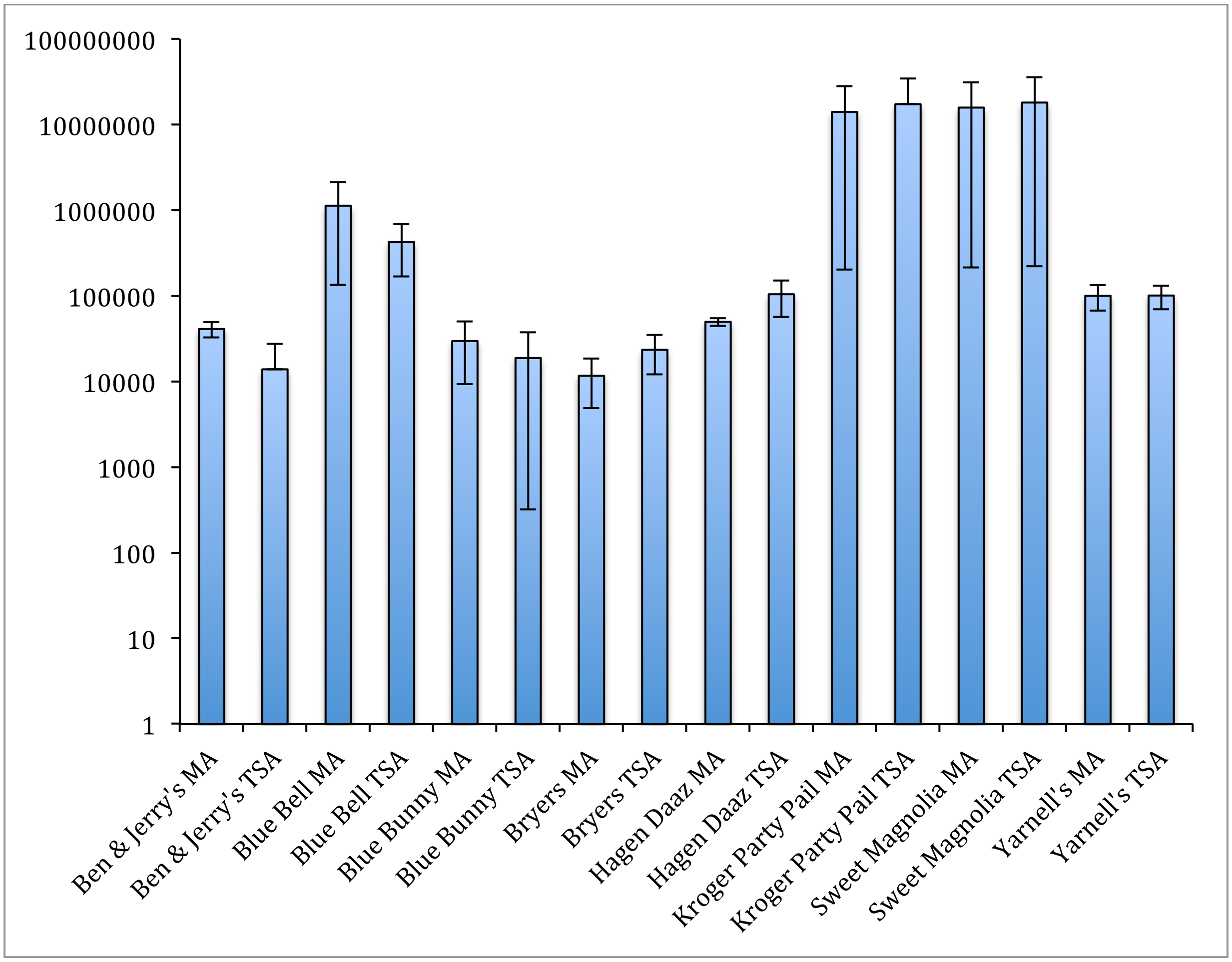


Figure 5: Average bacterial counts in different brands of ice cream (as determined from three different flavors) and expressed as CFU per pint carton. Counts were determined using milk agar (MA) and tryptic soy agar (TSA) plates, and are means (+/- SE) of three plates.

yielded the least CFUs (Fig. 6). However, there was no statistically significant difference in bacterial counts across the three flavors (p-values of 0.15 to 0.20 for all comparisons), likely because of the high amount of variability between different brands.

Bacteria Identification

The presence of DNA in each extraction was confirmed through DNA gel electrophoresis (Fig. 7), and a portion of the 16S rRNA gene in each sample was subsequently sequenced. Sequences classified as the Firmicutes phylum were the most commonly found, accounting for 86% of sequences detected across all brands and flavors (Fig. 8). Other phyla detected included Actinobacteria, Bacteroidetes, and Proteobacteria. The greatest number of bacterial species were found in the Blue Bell and Blue Bunny brands, with seven distinct bacterial populations each (Fig. 9-10). Bacterial species identified in the Blue Bell brand included *Bacillus cereus* (48%), *Bacillus clausii* (19%), *Brevundimonas vesicularis* (11%), and *Acinetobacter guillouiae* (11%) (Fig. 9). The most common species identified in Blue Bunny brand were *Paenibacillus lautus* (67%) and *Bacillus humi* (23%) (Fig. 10). The Bryers brand yielded six different identifiable bacterial species including *Bacillus humi* (73%) and *Escherichia coli* (17%) (Fig. 9). The least diverse bacterial samples were obtained from the Kroger Party Pail brand, with only two identifiable species: *Bacillus cereus* (99%) and *Escherichia coli* (1%) (Fig. 11). *Bacillus cereus* was identified in all samples except those from Sweet Magnolia brand. *Escherichia coli* was identified in Bryers, Blue Bunny, and Kroger Party Pail brand samples (Fig. 9-12). Other commonly detected bacteria included *Bacillus clausii*, identified in four brands, and *Micrococcus luteus*, identified in three of the brands.

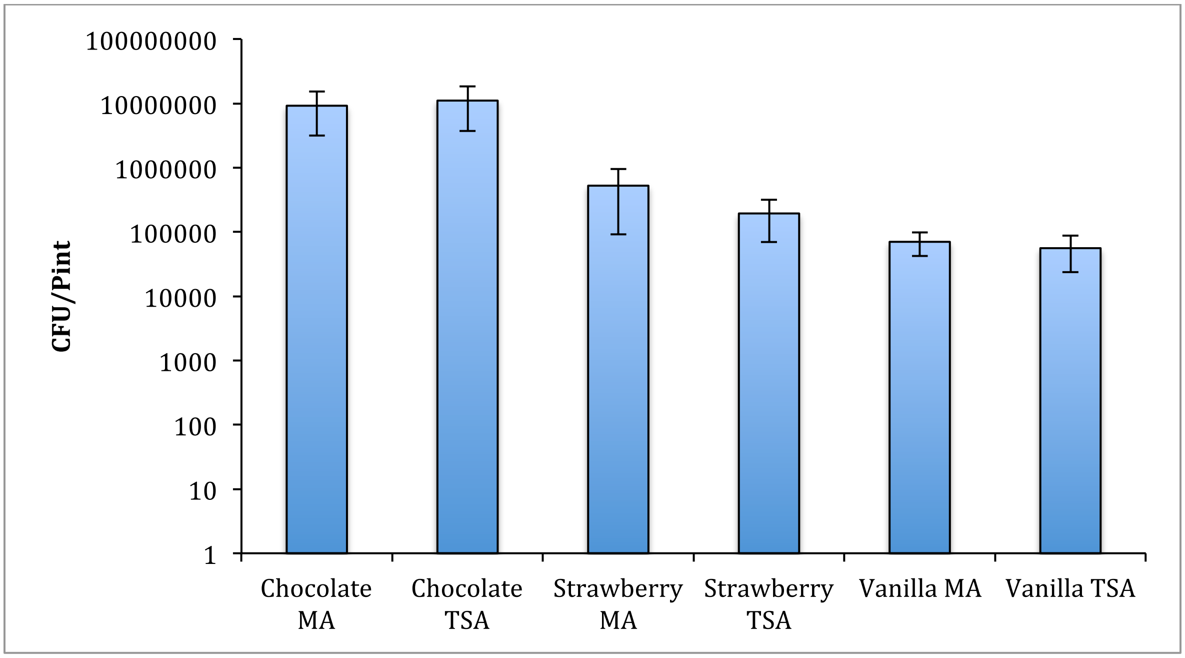


Figure 6: Average bacterial count in different flavors of ice cream when averaged across eight different brands. Counts are expressed as CFU per pint carton and were obtained using milk agar (MA) and tryptic soy agar (TSA) plates. Values are means (+/- SE) of three plates.

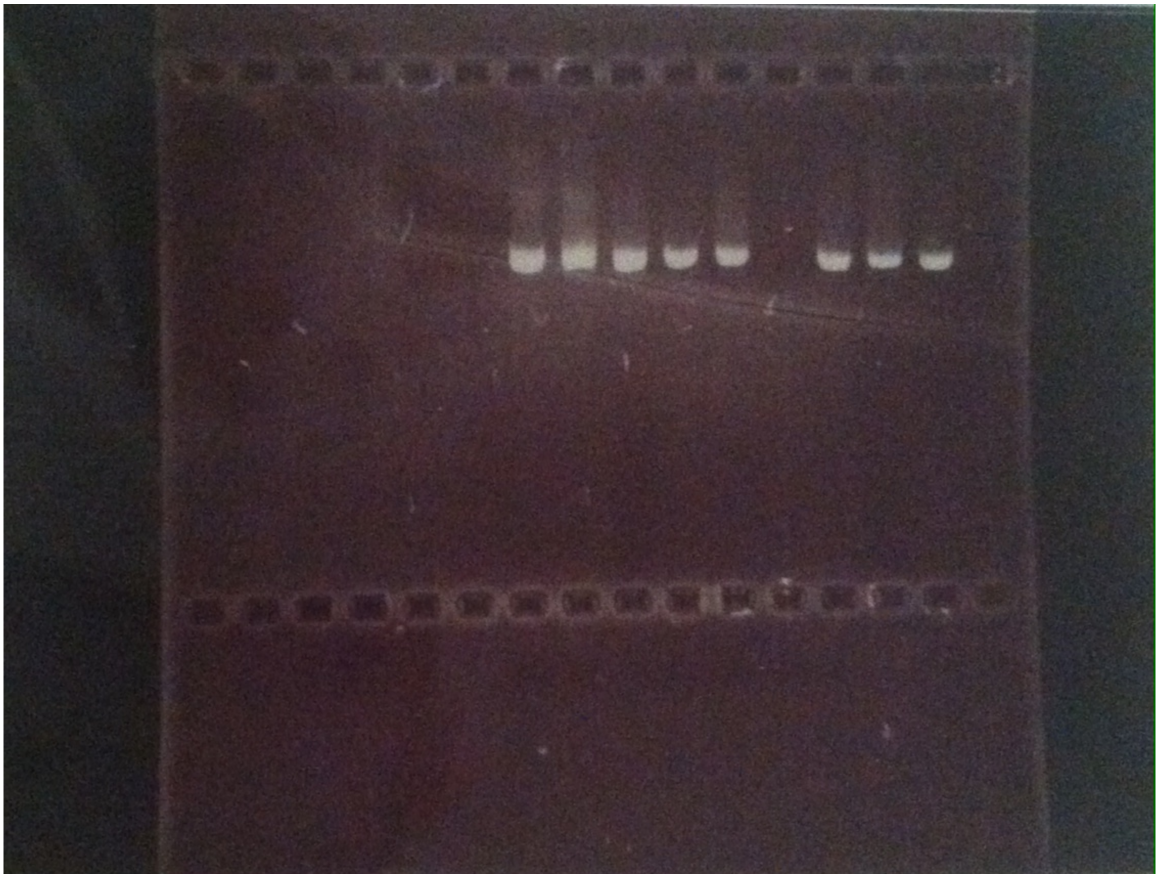


Figure 7: Results of DNA extraction from eight samples of ice cream cultured on either tryptic soy agar (TSA) or milk agar (MA) plates as confirmed via gel electrophoresis. DNA was extracted from Blue Bell vanilla, Blue Bell chocolate, Blue Bell strawberry, Hagen Daaz vanilla, Hagen Daaz chocolate, Hagen Daaz strawberry, Kroger Party Pail vanilla, Kroger Party Pail chocolate.

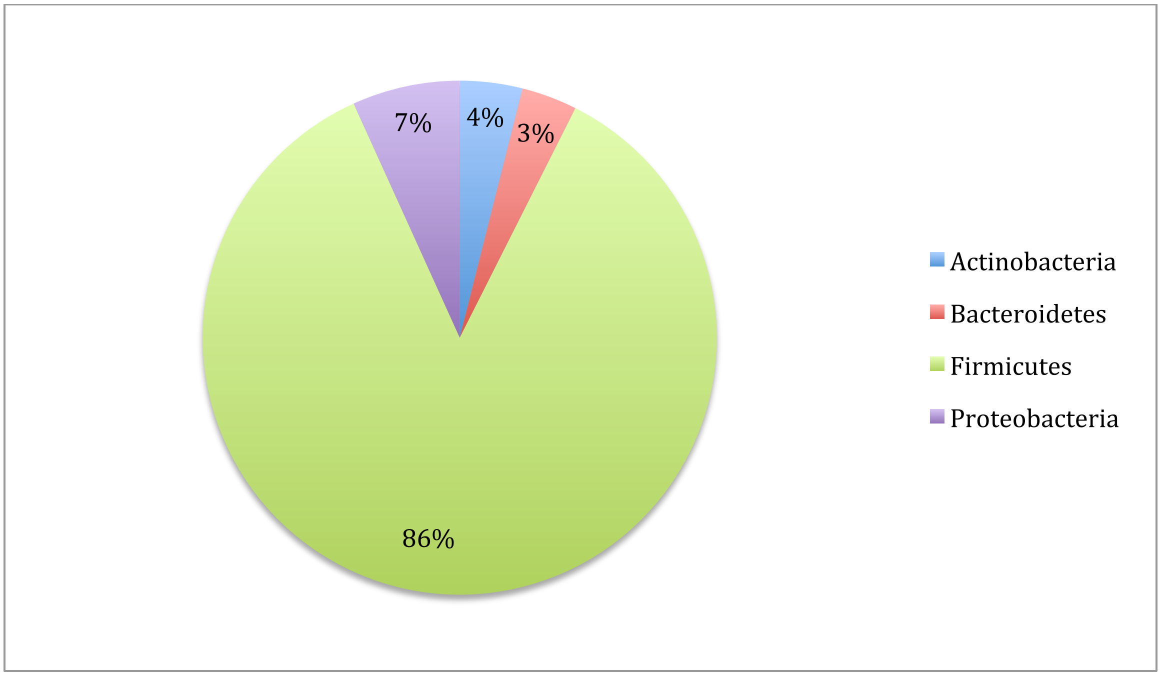


Figure 8: Bacteria from ice cream samples grown on tryptic soy agar (TSA) and milk agar (MA) were sequenced and identified. This figure shows the phylum makeup (% sequences obtained) across all samples.

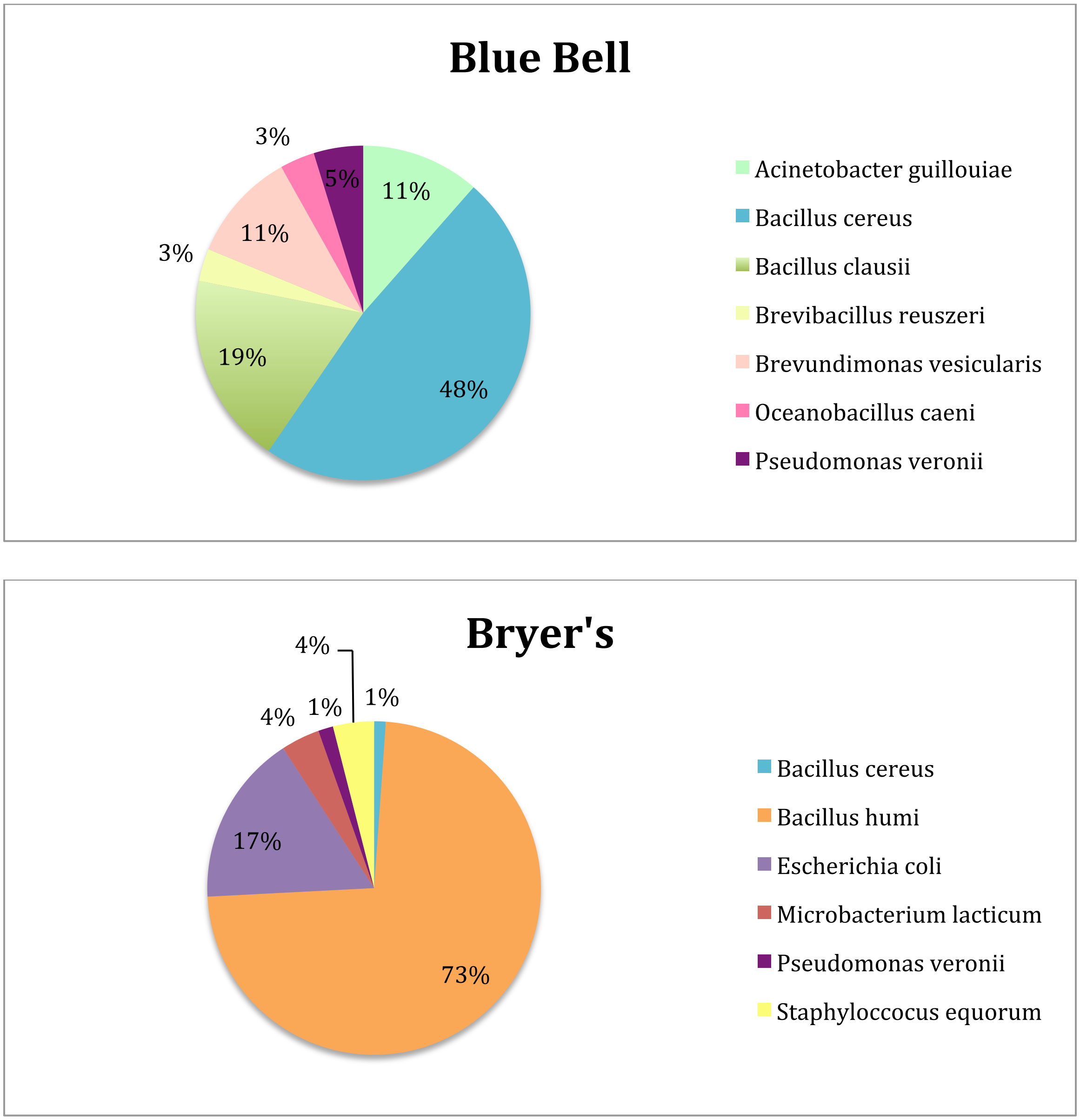


Figure 9: Bacteria from ice cream samples grown on tryptic soy agar (TSA) and milk agar (MA) were sequenced and identified. This figure shows the identifiable species makeup (% sequences obtained) from Blue Bell and Bryers samples.

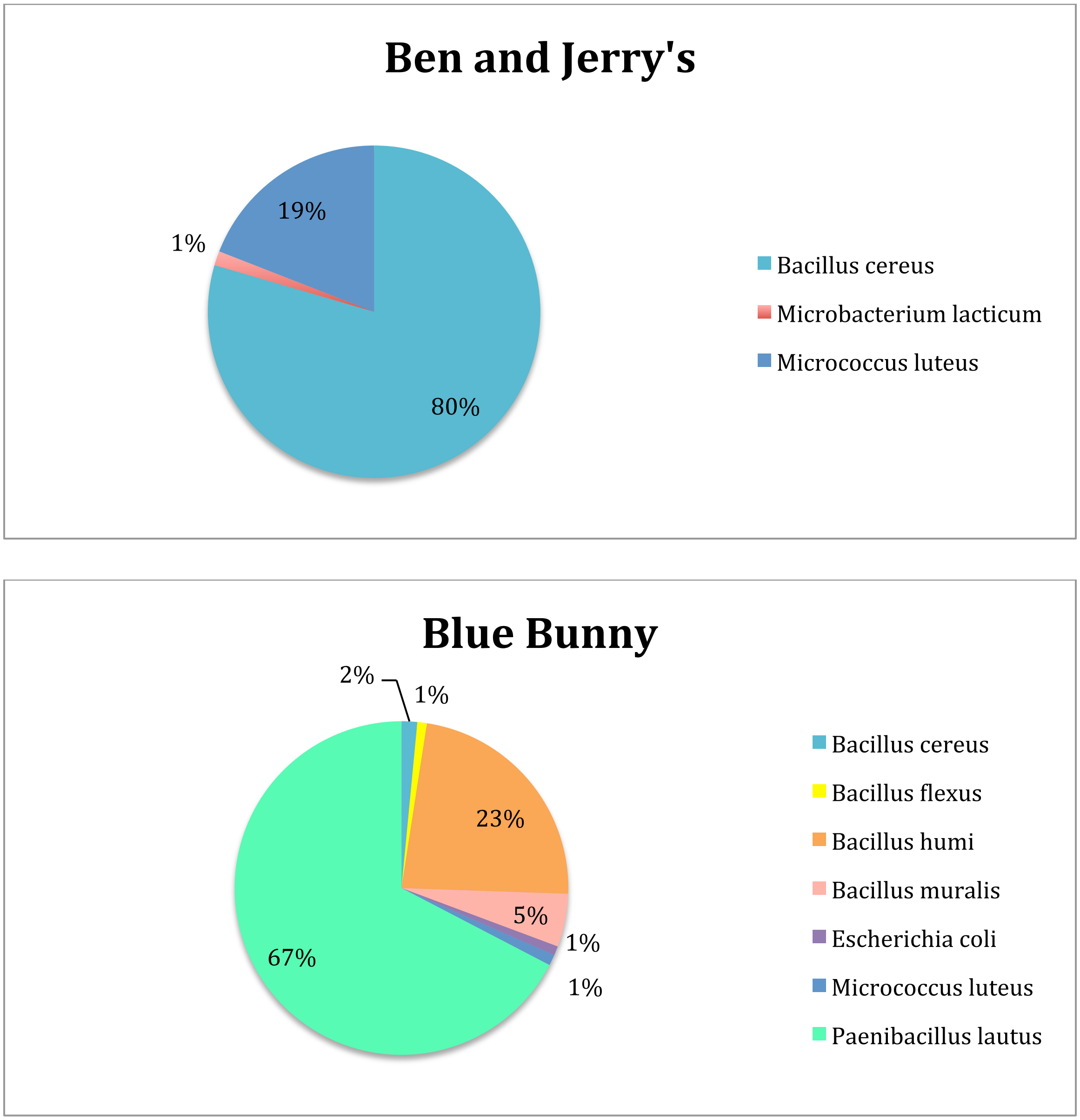


Figure 10: Bacteria from ice cream samples grown on tryptic soy agar (TSA) and milk agar (MA) were sequenced and identified. This figure shows the identifiable species makeup (% sequences obtained) from Ben and Jerry's and Blue Bunny samples.

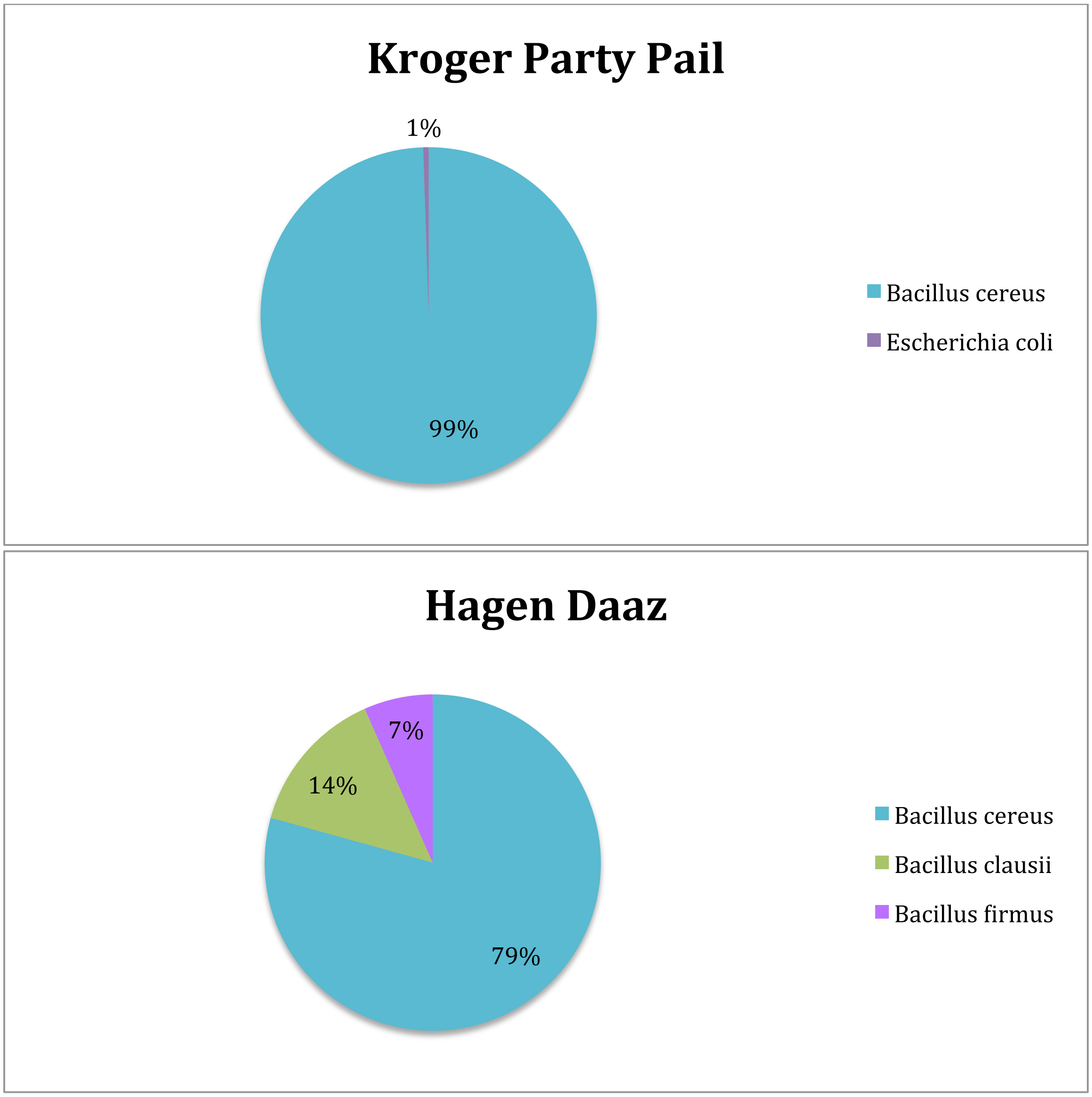
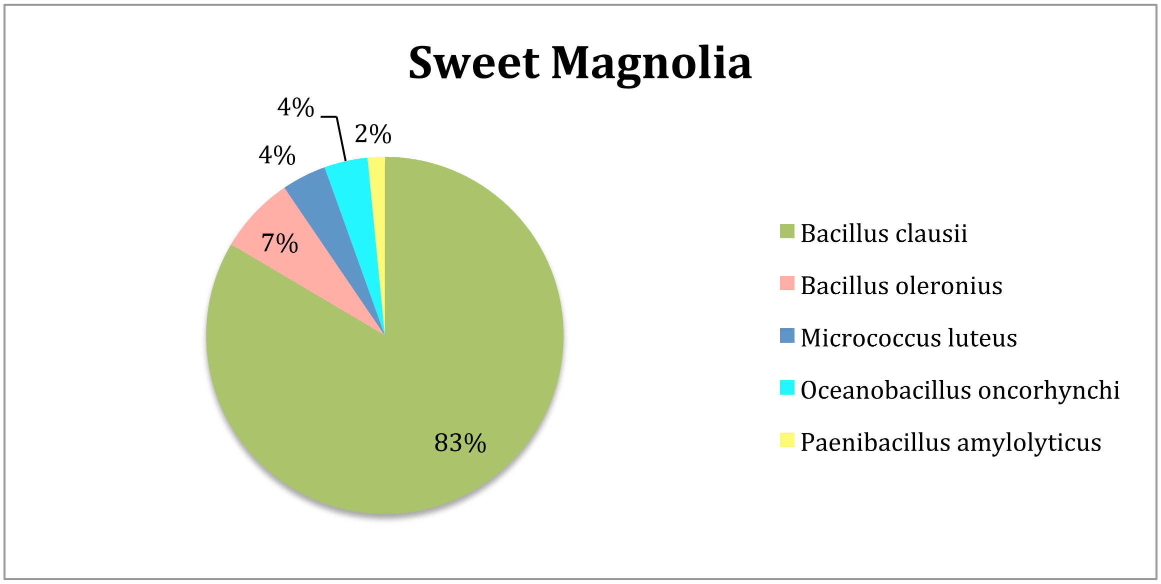


Figure 11: Bacteria from ice cream samples grown on tryptic soy agar (TSA) and milk agar (MA) were sequenced and identified. This figure shows the identifiable species makeup (% sequences obtained) from Hagen Daaz and Kroger Party Pail samples.



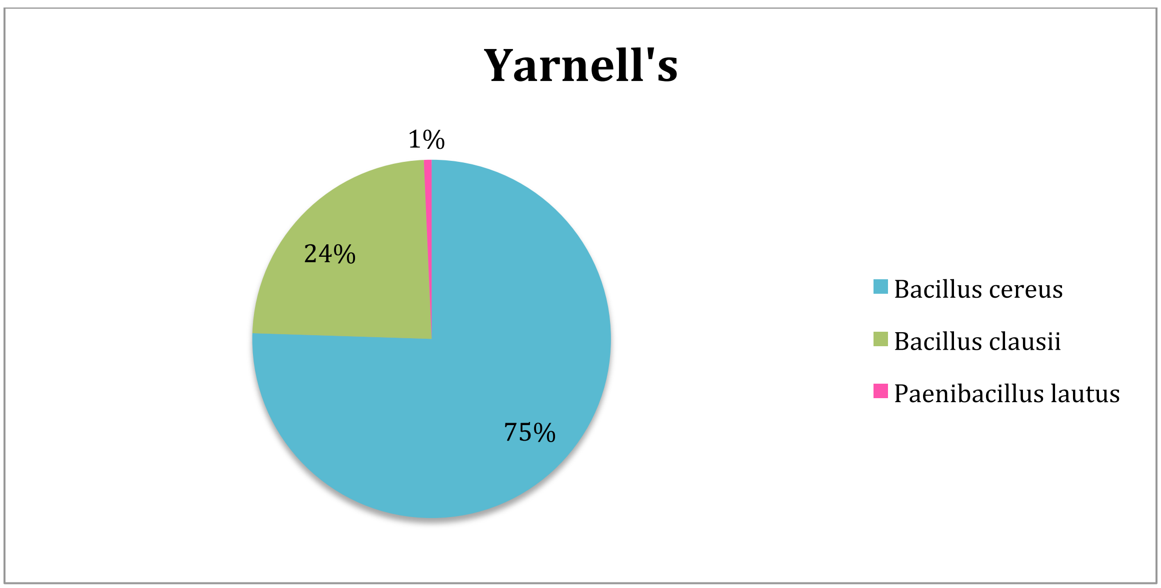


Figure 12: Bacteria from ice cream samples grown on tryptic soy agar (TSA) and milk agar (MA) were sequenced and identified. This figure shows the identifiable species makeup (% sequences obtained) from Sweet Magnolia and Yarnell's samples.

Bacillus humi, Microbacterium lacticum, Paenibacillus lautus, and Pseudomonas veronii were each identified in two different brands. All other species of bacteria detected were limited to one brand.

DISCUSSION

While most bacteria are harmless or even beneficial, pathogens found in food cause an estimated 55-105 million Americans to experience acute gastroenteritis each year, resulting in costs of $2-$4 billion annually. These numbers are likely an underestimate because many affected individuals do not seek treatment (Harris 2014). Although pasteurization of ingredients and freezing eliminates many bacterial hazards, ice cream could still potentially act as a vehicle for pathogen transmission. Some of the ingredients found in ice cream, including dairy products and eggs, are potential sources of bacterial contamination. Additionally, many brands of ice cream are now mass-produced and widely distributed, making any bacterial contamination capable of causing a widespread outbreak. For example, ice cream has been implicated in a number of widespread *Salmonella enteritidis* infections, with outbreaks in Florida in 1993 (CDC 1994), Georgia in 1994 (Mahon 1999), and across Minnesota, South Dakota, and Wisconsin in 1994 (CDC 1994). In April 2013, 13 people were infected with staphylococcal enterotoxins from eating ice cream at a hotel in Freiburg, Germany. Of these 13 cases, seven had to be hospitalized (Fetsch 2014). Most recently, in March 2015, a Texas Blue Bell factory stopped the production of eight ice-cream products that were linked *Listeria monocytogenes.* This bacterium causes Listeriosis, a rare but serious foodborne illness. Patients at a Kansas hospital were served Blue Bell

Creameries' prepackaged, single-serving ice cream products. Five of these patients became infected, and three of the cases proved fatal (Food and Drug Administration 2015). Therefore, although it may seem unlikely, ice cream is able to act as a vehicle for pathogen transmission and be a cause of outbreaks of foodborne illnesses.

Based on the numbers of CFUs obtained, this study predicts up to 4.5 million bacteria per ½ cup serving of ice cream, depending on the flavor and brand. My first hypothesis was that there would be higher bacterial counts in chocolate and strawberry ice cream compared to vanilla ice cream, because of increased numbers of ingredients and perhaps lower quality milk being used in the manufacture of those flavors. To some extent, this study supports that hypothesis. Averaging all eight brands, the milk agar (MA) plates inoculated with samples from chocolate ice cream had a 128-fold higher CFU count than those from vanilla ice cream samples. The strawberry ice cream samples cultured on the same type of plates yielded nearly seven times as many CFUs as the vanilla samples. Similar results were seen using TSA plates with chocolate samples generating 197 times more CFUs than vanilla, and strawberry samples giving three times as many CFUs on TSA plates as the vanilla samples. Whether the cause of these higher counts reflects more ingredients (and therefore a higher chance of contamination) or lower quality ingredients cannot be determined, but it may well be a combination of both factors. However, while these numbers are certainly suggestive of more bacteria in chocolate flavors versus strawberry or vanilla, the variation in counts between brands means that the differences in these numbers were not statistically significant, giving p-values of 0.15 to 0.20.

My second hypothesis was that the farmer's market local ice cream would contain a greater number of bacteria than the commercial brands, because of reduced processing and less regulation. This study partially supports this hypothesis. The Sweet Magnolia farmer's market brand yielded high numbers of CFUs, particularly in the chocolate samples tested. Averaging the three plates tested for each flavor, the only brand that yielded higher CFUs in the chocolate flavored samples was Kroger Party Pail brand, a budget brand that might be expected to contain lower quality ingredients. Samples of vanilla flavored Sweet Magnolia brand yielded the highest numbers of CFUs for any of the vanilla flavors tested across all eight brands. Because, unlike all the other brands tested, it is not nationally distributed there is likely considerably less regulation on this farmer's market brand. Except for two grocery stores, all the retailers of Sweet Magnolia are farmer's markets, restaurants, or inns.

Besides the farmer's market brand, seven other commercial brands were tested. Of those, Ben and Jerry's, Bryers, and Hagen Daaz brands were sealed with a plastic covering around the lid. This covering may have helped prevent bacterial contamination during transport because all three of these brands yielded low CFUs across all flavors. Ben and Jerry's strawberry and vanilla flavors did not yield any visible colonies on TSA plates, and MA plate counts were low. Hagen Daaz yielded low counts on both types of plates, especially the vanilla and chocolate flavor samples. Bryers ice cream had the lowest bacterial counts of any brand studied. Blue Bell, Blue Bunny, and Yarnell's brands had higher counts of bacteria than these three, but less than the farmer's market brand or the cheaper, bulk brand (Kroger Party Pail). The lids of these five brands were not as well protected, as they did not have a plastic seal under the lid.

It seems that, in addition to the number and quality of ingredients, packaging is an important factor for the prevention of bacterial contamination. One point that should be considered when evaluating these apparent differences between brands is that it could also represent variation from one container to another. My sampling was limited to just one container from each brand, and while differences in bacterial counts might appear to be differences between brands, it could also be just differences between containers. Examining multiple, replicate containers from each brand would clarify this, but that was beyond the scope of this study.

Member of the phylum Firmicutes were the most commonly detected bacteria in the cultures. More specifically, *Bacillus*, a genus of spore-forming Firmicutes, was the most common genus found in the sequencing libraries, and various species (e.g. *B. cereus, B. clausii, B. firmus, B. flexus, B. humi, B. oleronius*) were detected. While the presence of a high proportion of *Bacillus*-like sequences could reflect either a bias for those organisms to grow during culture or a bias towards them during DNA extraction and sequencing, it is interesting as this genus has health implications. Many species in this genus produce secondary chemicals or form endospores, which can be troublesome for food producers.

At least one *Bacillus* species*, B. cereus,* is a well-known cause of food poisoning. *B. cereus* was found in all brands of ice cream tested. This species is an opportunist pathogen and an acknowledged problem for the dairy industry (Helgason 2000). Wide ranges of infections have been reported from this pathogen, both in immune-compromised and healthy patients. The *B. cereus* endospores can survive normal cooking procedures and can multiply and produce toxins if the food is stored improperly (Logan 2012). That *B. cereus* was present in all of the brands tested, is a little alarming as it demonstrates that this organism is widely distributed in ice cream, and consumers could at any point be at risk of exposure.

However, not all species of *Bacillus* are necessarily a problem; *Bacillus clausii* was detected in Blue Bell, Hagen Daaz, Sweet Magnolia, and Yarnell's brands. *B. clausii* is a probiotic that can benefit human health. This species has been widely used in Italy since the 1960s for treatment of viral diarrhea in children and for antibiotic related side effects (Nista 2004). Thus, not all of the bacterial cultures detected in this study may be harmful, and as with other dairy products (yogurt etc.), ice cream may sometimes contain beneficial probiotic bacteria.

Escherichia coli were found in Blue Bunny, Bryers, and Kroger Party Pail samples. *E. coli* is found normally in human intestines and most strains are harmless. However, some strains can cause urinary tract infections, diarrhea, or infant mortality. One particular strain, 0157:H7, can cause severe or fatal renal or neurological problems. Pathogenic strains of *E. coli* produce enterotoxins that can cause diarrhea and vomiting by causing fluid to be secreted into the intestines (Berg 2004). During October 2007, an *E. coli* outbreak infected twelve children after eating ice cream produced at a farm in Antwerp, Belgium. The *E. coli* strains were found in soiled straw samples from the farm, fecal samples from the farm's calves, and fecal samples from the patients (De Schrijver 2008). Therefore, it's not unreasonable to imagine that locally produced farmers market type brands of ice cream could harbor potentially harmful strains of *E. coli.*

Micrococcus luteus was found in Ben and Jerry's, Blue Bunny, and Sweet Magnolia brands. *M. luteus* is not considered a human pathogen, but some studies have linked this bacterium to septic shock (Albertson 1978). Recently, in Japan, a case of septic shock by *M. luteus* was reported in an autologous hematopoietic stem cell transplant recipient. The 67-year-old had a catheter implanted for 12 days, which most likely caused the infection (Korgure 2014). Foodborne cases of septic shock from *M. luteus* are probably unlikely, but, as with some of the other bacterial species, the presence of this potentially harmful bacterium in three different brands of ice cream is still disturbing.

Additional studies (more replication, sampling across a larger geographic area etc.) are needed for conclusive results, but consumers should probably be aware of potentially higher bacterial counts in chocolate ice cream compared to strawberry or vanilla flavors. It may be that ice cream producers use more ingredients in the manufacture of these flavors or utilize lower quality milk because the additional added flavors can disguise the taste. Either could result in an increased bacterial load. Consumers should also be aware of the increased presence of bacteria in locally produced "farmer's market" ice cream, most likely a result of reduced processing and looser regulations. That said, it is important to keep in mind that most of the bacterial species found were harmless or even beneficial, although some potential pathogens were also detected. Consumers, especially those with compromised immune systems should be aware of the potentially increased risk of foodborne illness when choosing chocolate or locally produced brands of ice cream.

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